REMARKS

Claims 1-36 are pending in the present application. Claims 5-8 are rejected. Claims 1-4 and 9-36 are withdrawn from consideration as being drawn to a non-elected invention.

Objection to the Claims

The Examiner objects to claims 6 and 8 as not being narrower than the claims they depend from. Applicants herein change the claims to recite "further comprising" thereby obviating the rejection.

Rejection under 35 USC 112, first paragraph

Applicants were mostly successful in overcoming the rejections based upon lack of enablement and written description in the last Amendment. However, the Examiner maintains the rejection of claims 5-8 as not properly enabled based upon the rationale that the specification fails to teach "how to use" this broad genus of peptides. This is based upon the rationale that there is a large genus, there is no identification of a ligand for most of the peptide species, and there is no identification of a specific biological function for most of the peptide species. Further, the Examiner says that one of ordinary skill in the art would not know which bromodomain peptides would bind to which acetyl lysine containing peptides. Applicants respectfully contend that the Examiner incorrectly dismisses the supplemental evidence provided regarding the bromodomains of other proteins.

<u>Regarding Enablement</u>

The Examiner maintains the rejection of claims 5-8 as allegedly not being enabled. The Examiner notes that these claims read on peptides comprising the ZA loop of protein bromodomains that fall within an indicated generic structure, that of SEQ ID

NO: 3. The claims also recite the functional limitations that the peptides are useful for screening for inhibitors of interaction between a bromodomain and an acetylated lysine.

Applicants previously submitted that because teachings regarding the potential use of the P/CAF bromodomain are explicitly set forth, those skilled in the art would be able to use any ZA loop of any bromodomain.

Applicants respectfully submit that the patent law does not require any number of species to describe a genus. Nonetheless, Applicants previously submitted the Declaration of Dr. Ming-Ming Zhou pursuant to 37 C.F.R. 1.132 wherein the Declarant clarified that he is aware of many proteins containing a bromodomain that have been shown to interact with other proteins. Representative examples include the bromodomain of WSTF (Williams syndrome transcription factor) that interacts with lysine-acetylated histones (Fujiki, R., et al., *Ligand-induced transrepression by VDR through association of WSTF with acetylated histones.* Embo J, 2005); the bromodomain of the transcriptional cofactor p300 that binds to nucleosome (Ragvin, A., et al., *Nucleosome binding by the bromodomain and PHD finger of the transcriptional cofactor p300.* J Mol Biol, 2004. **337**(4): p. 773-88); the bromodomain of CBP/p300 that binds to acetylated MyoD (Polesskaya, A., et al., *Interaction between acetylated MyoD and the bromodomain of CBP and/or p300.* Mol Cell Biol, 2001. **21**(16): p. 5312-20); the bromodomain of NoRC (the SNF2h-containing chromatin-remodeling complex) that interacts with K16-acetylated histone H4 (Zhou, Y. and I. Grummt, *The PHD finger/bromodomain of NoRC interacts with acetylated histone H4K16 and is sufficient for rDNA silencing.* Curr Biol, 2005. **15**(15): p. 1434-8); the bromodomains of BDF1 and BDF2 that bind to histone H4 (Matangkasombut, O., et al., *Bromodomain factor 1 corresponds to a missing piece of yeast TFIID.* Genes Dev, 2000. **14**(8): p. 951-62); the bromodomain of the WBSCR9 gene, encoding a novel transcriptional regulator, in the Williams-Beuren syndrome deletion at 7q11.23 (Peoples, R.J., et al., *Identification of the WBSCR9 gene, encoding a novel transcriptional regulator, in the Williams-Beuren syndrome deletion at 7q11.23.* Cytogenet Cell Genet, 1998. **82**(3-4): p. 238-46); the bromodomain-containing TIF1α: a possible link between KRAB zinc finger proteins and nuclear receptors (Le Douarin, B., et al., *TIF1alpha: a possible link between KRAB zinc finger proteins and nuclear receptors.* J Steroid Biochem Mol Biol, 1998. **65**(1-6): p. 43-50); and, the bromodomain

of CBP that interacts with human tumor suppressor p53 at acetylated lysine 372 (Mujtaba, S., et al., *Structural mechanism of the bromodomain of the coactivator CBP in p53 transcriptional activation.* Mol Cell, 2004. **13**(2): p. 251-63). (*See*, Paragraph 5) In view of this wealth of information in the art, it is clear that one of skill in the art has a wealth of bromodomains at his or her disposal. As such, a skilled artisan may practice the invention without undue experimentation.

Dr. Ming-Ming Zhou, in the Declaration previously submitted pursuant to 37 C.F.R. 1.132, clarified that he is aware of many proteins containing a bromodomain that have been shown to interact with other proteins and for which the consequence of this interaction is understood as regards biological activity. Examples of these include that the bromodomain containing 2 (Brd2) is expressed in distinct patterns during ovarian folliculogenesis independent of FSH or GDF9 action (Trousdale, R.K. and D.J. Wolgemuth, *Bromodomain containing 2 (Brd2) is expressed in distinct patterns during ovarian folliculogenesis independent of FSH or GDF9 action.* Mol Reprod Dev, 2004. **68**(3): p. 261-8); the bromodomain of the MLL-CBP fusion protein is required for generating a myelodysplastic-like syndrome that evolves into myeloid leukemia (Lavau, C., et al., *Chromatin-related properties of CBP fused to MLL generate a myelodysplastic-like syndrome that evolves into myeloid leukemia.* EMBO J., 2000. **19**: p. 4655-4664); the bromodomain-containing histone H3 acetylase dGcn5 is a key player in Drosophila melanogaster metamorphosis(Carre, C., et al., *The histone H3 acetylase dGcn5 is a key player in Drosophila melanogaster metamorphosis.* Mol Cell Biol, 2005. **25**(18): p. 8228-38); the bromodomain protein Brd4 is a positive regulatory component of P-TEFb and stimulates RNA polymerase II-dependent transcription (Jang, M.K., et al., *The bromodomain protein Brd4 is a positive regulatory component of P-TEFb and stimulates RNA polymerase II-dependent transcription.* Mol Cell, 2005. **19**(4): p. 523-34); the PHD finger/bromodomain of NoRC interacts with acetylated histone H4K16 and is sufficient for rDNA silencing (Jang, M.K., et al., *The bromodomain protein Brd4 is a positive regulatory component of P-TEFb and stimulates RNA polymerase II-dependent transcription.* Mol Cell, 2005. **19**(4): p. 523-34); the bromodomain-containing protein Bdf1p acts as a phenotypic and transcriptional multicopy suppressor of YAF9 deletion in yeast (Bianchi, M.M., et al., *The bromodomain-containing protein Bdf1p acts as a*

phenotypic and transcriptional multicopy suppressor of YAF9 deletion in yeast. Mol Microbiol, 2004. **53**(3): p. 953-68); Bdfl bromodomains' interactions with acetylated H4 tails help anchor the transcriptional protein complex TFIID to the promoter during the initial stages of transcription activation (Martinez-Campa, C., et al., *Precise nucleosome positioning and the TATA box dictate requirements for the histone H4 tail and the bromodomain factor Bdf1.* Mol Cell, 2004. **15**(1): p. 69-81); the CBP bromodomain and nucleosome targets are required for Zta-directed nucleosome acetylation and transcription activation (Deng, Z., et al., *The CBP bromodomain and nucleosome targeting are required for Zta-directed nucleosome acetylation and transcription activation.* Mol Cell Biol, 2003. **23**(8): p. 2633-44); the bromodomains anchor chromatin-modifying complexes to promoter nucleosomes (Hassan, A.H., et al., *Function and selectivity of bromodomains in anchoring chromatin-modifying complexes to promoter nucleosomes.* Cell, 2002. **111**: p. 369-379); the bromodomain mediates transcriptional intermediary factor 1alpha -nucleosome interactions (Remboutsika, E., et al., *The bromodomain mediates transcriptional intermediary factor 1alpha -nucleosome interactions.* J Biol Chem, 2002. **277**(52): p. 50318-25). (*See*, paragraph 7)

Dr. Ming-Ming Zhou, in the Declaration pursuant to 37 C.F.R. 1.132, clarified that he is aware of many proteins that have been shown to interact with the bromodomain of another protein. Representative examples include nucleosomal core histones H3, H4, H2A and H2B, each of which has multiple known lysine acetylation sites. In addition, other proteins including cellular proteins of p53 (Mujtaba, S., et al., *Structural mechanism of the bromodomain of the coactivator CBP in p53 transcriptional activation.* Mol Cell, 2004. **13**(2): p. 251-63); NF-κB (Greene, W.C. and L.F. Chen, *Regulation of NF-kappaB action by reversible acetylation.* Novartis Found Symp, 2004. **259**: p. 208-17; discussion 218-25) and HIF1α (Chun, Y.S., et al., *Phorbol ester stimulates the nonhypoxic induction of a novel hypoxia-inducible factor 1alpha isoform: implications for tumor promotion.* Cancer Res, 2003. **63**(24): p. 8700-7) interact with a bromodomain of another protein. (*See*, paragraph 6) In view of this wealth of information in the art, it is clear that one of skill in the art has a wealth of bromodomains and their corresponding ligand at his or her disposal. As such, a skilled artisan may practice the invention without undue experimentation.

As regards the acetyl-lysine that is bound, Applicants again refer to the Declaration of Dr. Ming-Ming Zhou pursuant to 37 C.F.R. 1.132 wherein the Declarant clarified that as reported in the specification, some bromodomains may not bind to the free amino acid acetyl-lysine alone. This may be due to the charged amino and/or carboxyl groups of the amino acid lysine that are adjacent to its acetyl moiety. However, bromodomains do in fact interact with and bind to an acetyl-lysine residue when it is presented in a polypeptide sequence such as those in proteins. In latter cases, these charged groups may be naturalized due to polypeptide connectivity. Hence, the acetyl lysine may be necessary for bromodomains to bind to a particular portion of a protein. (*See*, paragraph 8)

Regarding Written Description

The application provides several examples of peptides falling within the scope of SEQ ID NO.: 3. The application also provides examples of ligands for two of these peptides. Of the disclosed peptides, one is disclosed as useful for identifying inhibitors of HIV replication (the P/CAF peptide), and one as potentially useful for the inhibition of cancers (the CBP peptide).

Applicant respectfully submits that support for the role of the bromodomain and its interaction with the acetyl lysine of the Tat protein in HIV can be found on page 21, lines 18-30. More importantly, the application provides evidence that acetylated lysine 50 of Tat specifically binds to the bromodomain of P/CAF. The Examiner's attention is drawn to Figures 5-10 and the results of these experiments, which are shown on page 77. This information, taken together with the fact that Tat is tightly regulated by lysine acetylation, and that HIV-1 Tat transcriptional activity is absolutely required for productive HIV viral replication is supportive of a role for this bromodomain as a drug target for blocking HIV replication in cells.

A ligand for a bromodomain is defined on page 48, lines 22-23, wherein it states:

"A compound is identified as a potential ligand if it binds to the ZA loop of the bromodomain."

As shown on page 51, lines 25-28:

"In a particular embodiment of the present invention the bromodomain-ligand complex is the Tat-P/CAF complex and the compound identified by the screen can used to prevent, retard the progression, treat and/or cure AIDS."

Applicant further asserts that Example 1 on pages 52-62 supports the ZA loop of the bromodomain binding to its ligand, which in the matter of the present application is an acetylated lysine, such as that found in acetyl-histamine.

Furthermore, Applicants have provided previously in the Declaration under 37 CFR 1.132 additional support for compounds identified by the methods described herein. The Examiner's attention is drawn to the inventor's declaration whereby compounds have been identified on the basis of the bromodomain and ZA loop sequences and coordinates provided in the instant application. This represents yet further proof that Applicants have described how to use the invention.

Rejection under 35 USC 102

Applicants were mostly successful in overcoming the rejections based upon anticipation in the last Amendment. However, the Examiner maintains the rejection of claims 6 and 8 in part as anticipated by Denis and Green, *Genes Dev* 10(3):261-271. The rejection is based upon the "objection" as noted above and can be overcome by making claims 6 and 8 recite all of the recitations of claims 5 and 7 with the additional "further comprising" a fusion protein. Applicants herein make the change thereby obviating the rejection.

Fees

No fees are believed to be necessary in connection with this response. However, if this is in error, authorization is hereby given to charge Deposit Account No. 11-1153 for any underpayment, or credit any overages.

Conclusion

Applicants believe that the foregoing amendments to the claims place the application in condition for allowance. Withdrawal of the rejections is respectfully requested. If a discussion with the undersigned will be of assistance in resolving any remaining issues, the Examiner is invited to telephone the undersigned at (201) 487-5800, ext. 114, to effect a resolution.

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